Acta Derm Venereol 2013; 93: 693–700

CLINICAL REPORT

The Many Faces of Solitary and Multiple Erythema Migrans

Pernilla ERIKSSON[1], Marika T. SCHRÖDER[1], Kirsi NIIRANEN[1], Antti NEVANLINNA[2], Jaana PANELIUS[1] and Annamari RANKI[1]
[1]Department of Dermatology and Allergology, Helsinki University Central Hospital, and [2]Center for Information Technology, University of Helsinki, Helsinki, Finland

Case definitions for European Lyme disease have been published. However, multiple erythema migrans may pose a diagnostic challenge. Therefore, we retrospectively reviewed the clinical and serological findings and response to therapy in a cohort of consecutive 54 patients with PCR-confirmed erythema migrans, referred to a university dermatology clinic. The proportion of patients with multiple erythema migrans lesions (usually 2 or 3) was almost equal (46%) to the proportion of patients with single erythema migrans lesions (54%). All patients, except for 2 multiple erythema migrans patients with a concomitant autoimmune disease, completely responded to treatment. In conclusion, multiple erythema migrans may be more common than anticipated, and since only 50% of the patients were seropositive when seeking medical help, PCR testing of skin lesions is helpful to confirm the diagnosis in clinically atypical cases. ***Key words: Lyme borreliosis; Lyme disease; erythema migrans; multiple erythema migrans; Borrelia burgdorferi.***

Accepted Nov 5, 2012; Epub ahead of print Feb 28, 2013

Acta Derm Venereol 2013; 93: 693–700.

Pernilla Eriksson, Department of Dermatology and Allergology, Helsinki University Central Hospital, PO Box 160, FIN-00029 HUS, Finland. E-mail: pernilla.eriksson@fimnet.fi, annamari.ranki@hus.fi

In Europe, Lyme borreliosis (LB) displays the highest incidence in the north and central parts of Europe (Austria, Germany, Slovenia, Baltic states, Sweden and Finland) (1, 2).

Approximately 1,500 laboratory-confirmed LB infections are diagnosed annually in Finland according to the National Institute for Health and Welfare (3), but this number is an underestimate, since many patients with erythema migrans (EM) are treated on clinical grounds with no laboratory confirmation.

Almost 80% of all patients with LB due to *Borrelia burgdorferi* sensu lato have cutaneous skin lesions (4). The clinical hallmark lesion of early LB is EM, but multiple EM (MEM) lesions are also frequently seen in European patients (5–8). MEM can be an early sign of disseminated LB (5, 9). Early recognition of such manifestations is important in order to avoid evolution to systemic manifestations, such as neurological, ophthalmological, cardiac or rheumatic borreliosis. In addition, lymphocytoma can be caused by *B. burgdorferi*. Acrodermatitis chronica atrophicans (ACA) is a late skin manifestation of LB (10). Common systemic symptoms associated with skin manifestations of LB are typically arthralgia, muscle pain, fatigue, headache, fever, and nausea.

Recently, new case definitions for European LB have been published (2). However, the highly variable clinical manifestations of MEM may pose a clinical challenge and often need diagnostic support from laboratory tests.

We performed a retrospective review of a cohort of patients with LB with solitary or multiple EM confirmed with PCR-based detection of *B. burgdorferi* in skin lesions. We analysed the demographics and clinical presentation, with a special emphasis on the presence of systemic symptoms and serological response during the course of the disease, and evaluated the therapeutic outcome.

MATERIALS AND METHODS

Patients

We reviewed the data of all patients with PCR-confirmed *B. burgdorferi* infection seen at the Department of Dermatology at Helsinki University Central Hospital from 2008 to 2010. During this period, a total of 87 patients were PCR-positive for *B. burgdorferi* in the skin biopsy. Only those with solitary EM (SEM) and MEM were included in this study, this group consisted of 54 patients. Demographic data, clinical features, photographs, serology, and skin lesion histology were retrieved from patient files. Antibiotic therapy, dosage and length of treatment were reviewed, in addition to therapeutic outcome.

Borrelia antibodies

Anti-borrelial immunoglobulin (Ig)G and IgM antibodies were determined by 2 immunoassays. The screening test was based on an enzyme immunoassay using *B. afzelii* whole-cell lysate (strain PKo) as an antigen in the determination of IgM antibodies. In IgG antibody determinations, a variable surface antigen, VlsE (variable major protein-like sequence expressed) antigen from *B. afzelii* (strain PKo) was added (Genzyme Virotech GmbH, Russelsheim, Germany). For all positive screening tests, a confirming chemiluminescent immunoassay (Liaison®) was performed as a routine procedure using recombinant VlsE antigen obtained from *B. garinii* (PBi strain) for IgG antibodies and recombinant VlsE antigen combined with OspC (outer surface protein C) obtained from *B. afzelii* (PKo strain) for IgM antibodies (Diasorin, Saluggia, Italy) by HUSLAB (11). In the screening test, the cut-off for antibody positivity was titre level 9 (Virotech units), where 9–11 was borderline and > 11 was a positive result. In the confirming test, the cut-off was at titre

level 10 for IgG and 18 for IgM antibodies (10–15 was borderline for IgG and > 15 was positive; 18–22 was borderline for IgM and > 22 was positive). The antibody result was considered positive if IgG antibodies against VlsE were positive. If only IgM antibodies were slightly positive or the antibody titres were at the borderline level, the result was considered borderline. If the IgG and IgM antibodies were both at negative level, the result was considered negative.

PCR-based detection of B. burgdorferi *skin lesions*

A skin biopsy was obtained from the active margin area (erythema) of suspected lesions, snap frozen and stored at –70ºC. The DNA was extracted as previously described (12) and the radioactive OspA-specific probe originally used for hybridization confirmation, has been replaced with a non-isotopic label, digoxigenin (12). In addition to the OspA gene, the PCR amplification also covered the 23S and 16S ribosomal RNA genes (primer sequences and annealing temperatures provided on request). All amplifications were carried out with a MJ Research PTC-200 thermal cycler. After PCR, samples were analysed on a 1.5% agarose gel, the amplicons were transferred to nylon membranes and hybridized with the relevant probe, immunodetected with anti-digoxigenin-conjugated alkaline phosphatase (Roche Applied Science, Mannheim, Germany) and visualized with the chemiluminescence substrate CSPD (Boehringer Mannheim, Mannheim, Germany) on X-ray films.

Histopathology

Histopathological analysis was performed as a part of routine diagnostics in the Dermatopathology Laboratory of Helsinki University Central Hospital by an experienced dermatopathologist. Typically, in EM the epidermis is normal, but an inflammatory cell infiltrate consisting of lymphocytes and plasma cells is seen in the dermis around vessels and between the collagen bundles. The inflammatory cell infiltrate can be sparse or moderate and sometimes even extends to the subcutis.

Therapy

According to the recently updated evidence-based national guidelines for diagnosis and treatment of cutaneous bacterial infections (13), treatment of SEM and lymphocytoma is recommended via oral amoxicillin 500–1,000 mg 3 times a day or oral doxycycline 100–150 mg twice a day for 2–3 weeks. ACA and MEM are usually treated with ceftriaxone 2 g intravenously daily for 3 weeks (14), which is consistent with the guidelines of the European Concerted Action on Lyme Borreliosis (EUCALB). ACA and MEM can also be treated as recommended by EUCALB (15). Table I shows the general treatment recommendations used in Finland.

Statistical analysis

Data were analysed using SPSS (version 19.0.0.1; IBM SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2003 (Microsoft, USA) software. The determination of the *p*-values was based on Fisher's exact test and Pearson χ^2.

RESULTS

The mean age of the patients was 53 years (age range 15–84 years) and most of the patients were female. Table II shows the demographic data of the patients in this study. Importantly, only 24% (13/54) of patients in our study recalled having a tick bite. No statistical difference in recalling a tick bite was found between patients with SEM (8/28%) and those with MEM (5/20%; $p = 0.545$).

Table I. *General treatment recommendations of cutaneous Lyme borreliosis in Finland. The treatment of solitary erythema migrans (EM) and lymphocytoma is based on the evidence-based Finnish guidelines for diagnosis and treatment of cutaneous bacterial infections (13). In Finland acrodermatitis chronica atrophicans (ACA) and multiple EM (MEM) are usually treated with ceftriaxone (15) while amoxicillin is used for MEM in case ceftriaxone is contraindicated (14). ACA and MEM can also be treated with peroral antibiotics according to the European Concerted Action on Lyme Borreliosis (15)*

Skin lesion type	Medication	Dosage	Course, days
Erythema migrans	Amoxicillin[a]	500–1,000 mg ×3	14–21
	Doxycycline[a]	100–150 mg ×2	14–21
Lymphocytoma	Amoxicillin[a]	500–1,000 mg ×3	14–21
	Doxycycline[a]	100–150 mg ×2	14–21
Multiple erythema migrans	Ceftriaxone[a]	2000 mg ×1 (i.v.)	14–21
	Amoxicillin	500–1,000 mg ×3	14–30
	Doxycycline[a]	100–150 mg ×2	14–30
Acrodermatitis chronica atrophicans	Ceftriaxone[a]	2000 mg ×1 (i.v.)	21
	Amoxicillin	500–1,000 mg × 3	14–30
	Doxycycline[a]	100–150 mg ×2	14–30

[a]Consistent with the guidelines of the EUCALB (15).

Variation in the clinical presentation of SEM and MEM lesions and frequency of associated symptoms

Among the 54 consecutive patients, 29 presented with SEM (54%) and 25 with MEM (46%). Most of the patients with MEM lesions had only 2 or 3 lesions (13/25; 52%). The highest number of recorded MEM lesions was 10 lesions in one patient. The size of the skin lesions varied, with the widest skin lesions 20–50 cm in diameter.

Table II. *Selected characteristics of 54 patients with PCR-confirmed solitary or multiple erythema migrans*

Patients	
Male, *n* (%)	18 (33)
Female, *n* (%)	36 (67)
Age, years, mean (median) [range]	53 (56) [15–84]
Tick bite recalled	13 (24)
Erythema migrans	
Total number of patients with SEM–/SEM+	29 (54)
Patients with SEM–	21 (72)
Patients with SEM+	8 (28)
Total number of patients with MEM–/MEM+[a]	25 (46)
Patients with MEM–	16 (64)
Patients with MEM+	9 (36)
Patients with annular erythemas	25 (46)
Patients with homogeneous erythemas	18 (33)
Patients with diffuse light erythema	4 (7)
Patients with undefined type[b] of erythema	7 (13)

[a]Range 2–10 lesions. [b]No photographs available, no detailed description of the clinical appearance in the patient files.
SEM–: solitary erythema migrans without associated systemic symptoms; SEM+: solitary erythema migrans with associated systemic symptoms; MEM–: multiple erythema migrans without associated systemic symptoms; MEM+: multiple erythema migrans with associated systemic symptoms.

If the size was not mentioned in the files, the size was approximated on available photographs. As to the localization of the skin lesions, most patients (61%) presented with lesion(s) on the lower extremities (Table III).

The skin lesions were either annular (25/47) or homogeneous (18/47) among the 47 cases photographed or recorded in detail (Fig. 1). Only a diffuse light erythema was reported in 4/47 patients. Light scaling, itch, clinical appearance reminding of other skin lesions (herpes zoster-, erysipelas-, haematoma- or vasculitis-like lesions), absence of migration of the erythema, uneven borders of the erythema or atypical colour of the erythema(s) were considered as atypical symptoms of LB by the referring physicians. Typically, *B. burgdorferi* infection was not diagnosed in the primary healthcare, since the skin lesions had been present for varying periods of time and without an obvious preceding tick bite. Most patients ($n=29$) were referred to the Department of Dermatology by primary healthcare physicians with the diagnosis "dermatitis non specificata". Eleven of these 29 patients (38%) had received inadequate oral or local antimicrobials or topical steroids. The remaining 24 patients were suspected of having LB, and 21 of these patients were referred by their physicians for confirmatory tests and/or for treatment of LB. Two patients were referred by their physicians because of persisting LB skin lesions after adequate treatment. Six of the patients were referred from the Department of Infectious Diseases.

The results of the routine histological examination before PCR-confirmation were typical for LB in 50/52 (96%) patients (perivascular inflammatory cell infiltrate consisting of lymphocytes and plasma cells, occasionally also between the collagen bundles). In one case septal panniculitis had developed below the site of EM and in another one the histology showed granuloma annulare.

In this cohort, 28% (8/29) of patients with SEM also had systemic symptoms, including headache, arthralgia, joint stiffness, fatigue, vertigo, fever, skin or muscle pain, visual disturbances, paraesthesia, muscle spasms, and sensations of arrhythmia. These symptoms were slightly more common among patients with MEM (9/25, 36%), although this did not reach statistical significance (Fisher's exact test $p=0.566$).

Table III. *Locations of skin lesions in 54 patients with single or multiple erythema migrans*

Skin lesion type (Total *n*)	Lower extremities *n*	Trunk *n*	Upper extremities *n*	Head and neck *n*
SEM– (21)	11	5	3	2
SEM+ (8)	5	3	0	0
MEM– (16)	11	12	7	0
MEM+ (9)	6	6	6	0
Total (54)	33	26	16	2

SEM–: solitary erythema migrans without associated systemic symptoms; SEM+: solitary erythema migrans with associated systemic symptoms; MEM–: multiple erythema migrans without associated systemic symptoms; MEM+: multiple erythema migrans with associated systemic symptoms.

Borrelia serology is more often positive in MEM patients with systemic symptoms

Serological screening was performed, with certain exceptions, at the initial stage of presentation (in 48 patients), immediately after treatment (in 25 patients), and during follow-up 6–24 months after treatment (in 37 patients) (Fig. 2). IgG and IgM antibodies were determined with the screening test, and if positive, also with the confirmatory test. For SEM, serological testing was performed because of the atypical presentation and/or unusual delay of evolution, as recommended in recent guidelines (2).

Half of the patients (24/48; 50%), had positive serology at the initial stage. The highest positivity of *B. burgdorferi*-specific antibodies at the initial stage was found in MEM patients with associated systemic symptoms (71.4%). Surprisingly, the initial serology was most often negative among MEM patients without associated systemic symptoms (6/14; 43%) (Table IV). However, no statistically significant difference was found in the frequency of the serological results at the initial stage (Pearson χ^2 $p=0.440$). After adequate antimicrobial therapy, the antibody titres tended to decrease, although 7 patients still had positive serology 6–24 months after treatment (Fig. 2). Two of these patients had SEM and 5 had MEM. VlsE IgG-antibodies decreased by more than 50% in 6 of these 7 patients.

Comparable treatment responses in all patient groups

The antibiotic regimens used for patients with the different types of skin lesions are shown in Table V. As a result of antimicrobial treatment, 52 of 54 (96%) patients were cured of their skin lesions, 1/54 had partially persisting skin lesions, and 1/54 had persisting skin lesions for at least 17 months. The skin lesions were completely cured in all 29 patients with SEM. Slight hyperpigmentation remained in 2 SEM patients without associated systemic symptoms. Twenty-three of the 25 (92%) patients with MEM were completely cured.

Two patients with pre-existing autoimmune diseases (Table VI) experienced some skin symptoms even after the antibiotic treatment. Neither of these 2 patients had any systemic symptoms of LB. The first patient with partly persisting skin lesions had coeliac disease. She received treatment with doxycycline for 8 weeks. Her skin lesions persisted for 15 months post-treatment showing a lichenoid reaction histologically.

The second patient presented with MEM-like skin lesions on the same location 17 months post-treatment and had concomitant Sjögren's syndrome. He was treated with ceftriaxone for 3 weeks. PCR on the first skin biopsy, with lymphocytic infiltrates, was positive

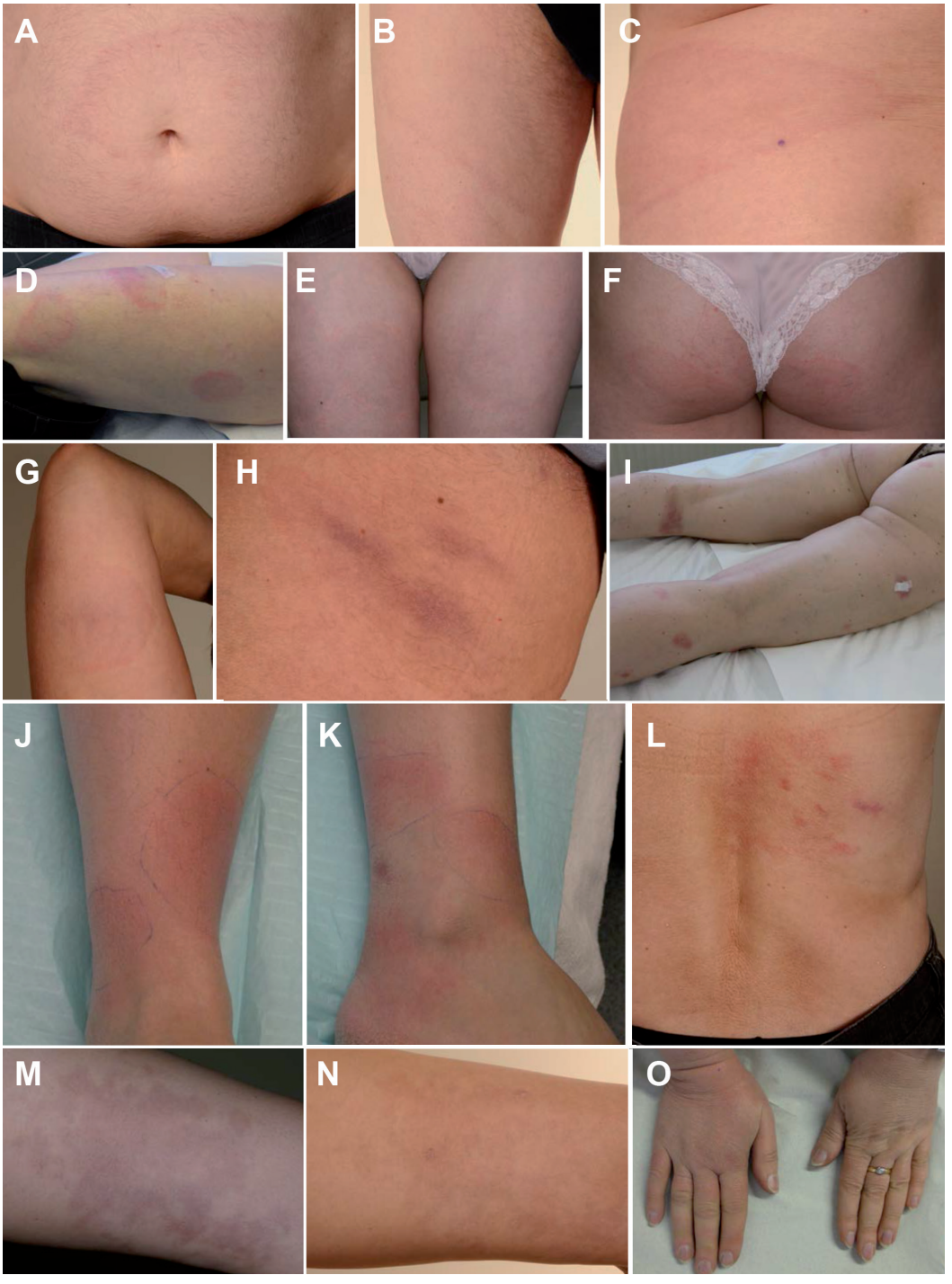

Fig. 1. Polymorphism of the clinical presentations of solitary erythema migrans (SEM) and multiple erythema migrans (MEM). The pictures are taken before antibiotic therapy unless stated otherwise. (A–C) Typical annular MEM lesions on several locations in a 53-year-old man. (D) MEM on the thigh of a 65-year-old woman. (E, F) MEM lesions on the thighs and buttocks of a 25-year-old woman. (G, H) MEM lesions on the upper arm and on the anterior upper thigh of a 43-year-old woman. (I) Erythematous patches of MEM on the legs in a 54-year old woman. (J, K) MEM lesions on the right ankle on a 40-year-old woman. (L) SEM on the back of a 64-year-old woman. The atypical clinical appearance might be a result of a preceding treatment with cephalexin. (M) SEM with somewhat lacy borders on the thigh of a 25-year-old woman. (N) The same skin area 2 years later, after amoxicillin therapy, with faint hyperpigmentation. (O) SEM of the right hand of a 54-year-old woman. Notice the oedema and redness on the distal part of the fingers and on the back of the right hand.

for 2 different *B. burgdorferi* genes, while a subsequent biopsy obtained 18 months later from a persisting skin lesion showed granuloma annulare histology and was weakly positive for the borrelia OspA gene only. Clinically, his skin lesions had changed from homogeneous erythematous lesions to annular lesions post-treatment. Two years previously he had had a clinically successfully treated EM on his back.

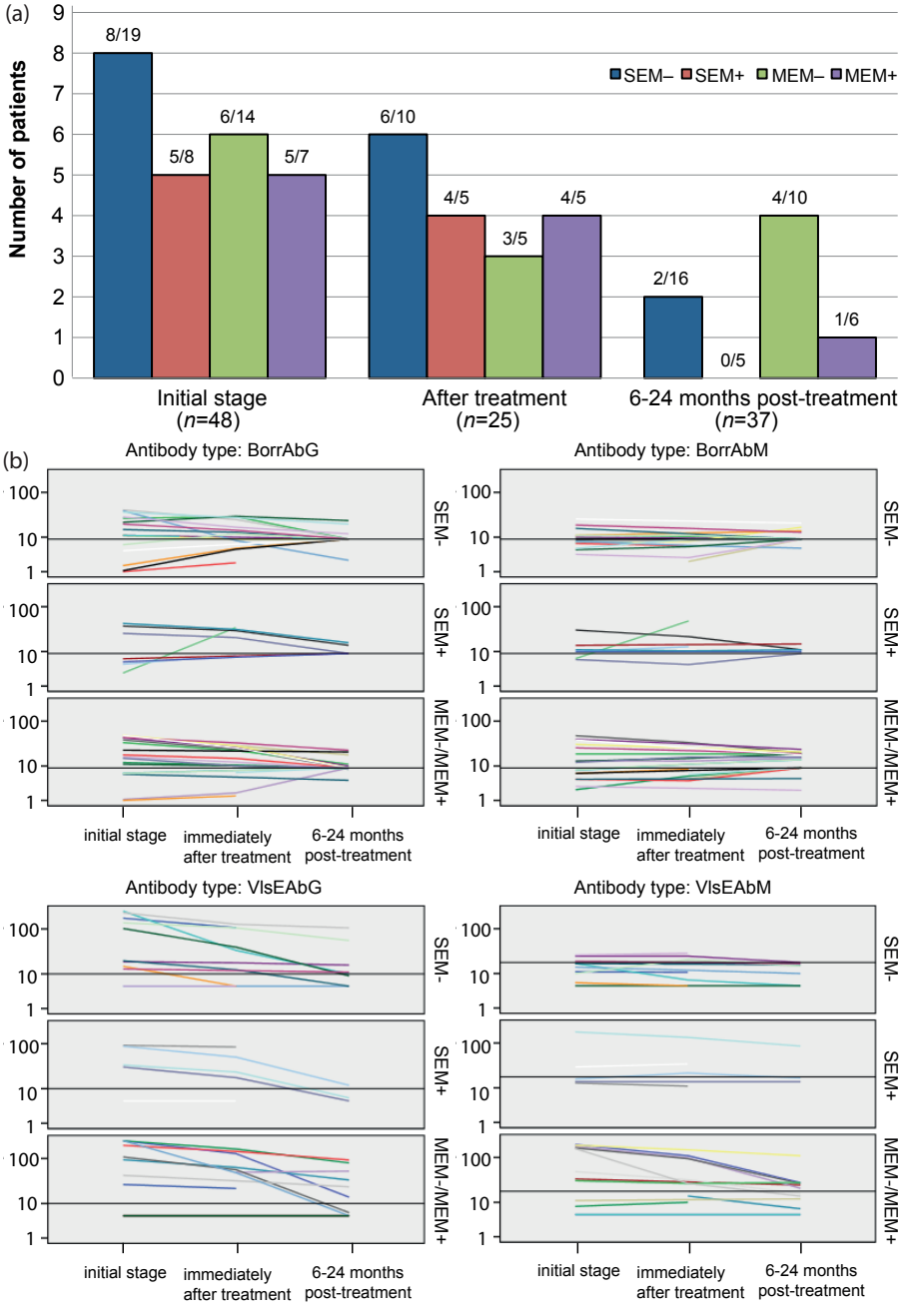

Fig. 2. (a) Serological positivity for Lyme borreliosis at different stages of treatment and follow-up. The number of patients with positive serological test result for *Borrelia* antibodies (the positivity was based on the final statement based on both IgG and IgM antibodies as described in the text) at 3 time-points in relation to treatment. Serological testing was performed at the initial stage (before treatment) in 48 patients, immediately after treatment in 25 patients and 6–24 months after treatment in 37 patients. The number after the slash (/) indicates the number of tested patients in each subgroup. The patients are divided into groups according to type of skin lesion and presence or absence of associated systemic symptoms, such as arthralgia, fever and fatigue for example. (b) IgG and IgM antibody levels at various stages of the disease, and after treatment. The cut-off for antibody positivity in the screening tests (BorrAbG, BorrAbM) was at titre level 9. In the confirming test, the cut-off was at titre level 10 for IgG (VlsEAbG) and 18 for IgM antibodies (VlsEAbM). SEM–: solitary erythema migrans without associated systemic symptoms; SEM+: solitary erythema migrans with associated systemic symptoms; MEM–: multiple erythema migrans without associated systemic symptoms; MEM+: multiple erythema migrans with associated systemic symptoms.

Table IV. *Serological results, based on 2 immunoassays at the initial stage*

	SEM– (*n*=19) *n* (%)	SEM+ (*n*=8) *n* (%)	MEM– (*n*=14) *n* (%)	MEM+ (*n*=7) *n* (%)	Total (*n*=48) *n* (%)
Positive	8 (42.1)	5 (62.5)	6 (42.9)	5 (71.4)	24 (50.0)
Borderline	6 (31.6)	2 (25.0)	2 (14.3)	0 (0.0)	10 (20.8)
Negative	5 (26.3)	1 (12.5)	6 (42.9)	2 (28.6)	14 (29.2)

SEM–: solitary erythema migrans without associated systemic symptoms; SEM+: solitary erythema migrans with associated systemic symptoms; MEM–: multiple erythema migrans without associated systemic symptoms; MEM+: multiple erythema migrans with associated systemic symptoms.

Altogether, 17/54 (31%) patients reported associated systemic symptoms during follow-up. Six of the 8 patients with associated systemic symptoms and SEM, were completely cured after antimicrobial therapy. The 2 patients with partially persisting systemic symptoms were both treated with amoxicillin or doxycycline and with preceding or following ceftriaxone 2 g intravenously for 3 weeks. One of them still experienced vertigo after treatment, although neuroborreliosis was ruled out, and the other had occasional idiopathic muscle spasms (normal brain magnetic resonance). Among the

Table V. *Treatment and treatment outcome of patients with solitary or multiple erythema migrans (SEM or MEM)*

Skin lesion type (*n*)	Patients *n*	Antibiotic	Outcome within 2 years
SEM (21)	12	Amoxicillin	Cured
	4	Doxycycline	Cured
	3	Amoxicillin and doxycycline subsequently	Cured
	2	Ceftriaxone	Cured
SEM with associated systemic symptoms (8)	2	Amoxicillin	Cured
	6	Ceftriaxone	Cured
MEM (16)	4	Amoxicillin	Cured
	5	Doxycycline	Cured: 4 Partially cured: 1
	7	Ceftriaxone	Cured: 6 Persisting: 1
MEM with associated systemic symptoms (9)	1	Amoxicillin	Cured
	1	Amoxicillin and doxycycline subsequently	Cured
	7	Ceftriaxone	Cured

Sixteen of the 22 patients who were treated with ceftriaxone also received treatments with amoxicillin and/or doxycycline. Eight of all of the patients had also previously been treated with inadequate antibiotics (cephalexin, cefuroxime) prior to diagnosis of Lyme borreliosis.

9 MEM patients with systemic symptoms, 5 were completely cured (from associated systemic symptoms), 4 still reported fatigue, joint or muscular pain. One of the latter showed a significant decrease in antibody levels compared with the first measurement. The other 3 had only slightly elevated antibody levels and showed no significant change during follow-up.

DISCUSSION

This study on Nordic patients with MEM confirmed with PCR from skin biopsies is the largest of its kind. According to our results, MEM may be more frequent in Europe than previously thought. The most recent case definition paper (2) does not state MEM frequency. However, according to several central European studies, MEM is expected to occur in 4–40% of the patients with EM (5–7). In our cohort, we observed a higher rate of EM patients with multiple lesions 25/54 (46%). One could ask whether the high prevalence of MEM in our cohort could be due to the assumption that MEM cases are more likely to be positive in the PCR confirmatory assay due to a higher number of microorganisms expected in these patients. However, the PCR assay we use is extremely sensitive, previously shown to detect 0.01 pg of Borrelia DNA (12) and, thus, not a likely explanation for a bias. On the contrary, the relatively high frequency of MEM may rather reflect the fact that many patients with classical SEM are not referred to a dermatology clinic, while MEM patients are more readily referred because of differential diagnosis difficulties.

Table VI. *Persisting skin lesions were more common among patients with autoimmune diseases*

	Cured *n*	Persisting *n*	Total *n*
Patients without autoimmune disease	46	0	46
Patients with autoimmune disease	6	2	8
Total	52	2	54

Screening for autoimmune diseases was not performed. Only pre-existing autoimmune diseases were recorded from the patient files.

In this study, 50% of patients with SEM or MEM were seropositive at the initial stage. Twenty-five were re-tested immediately after treatment, and 68% tested positive, showing that in some initially-seronegative patients seroconversion occurs during antimicrobial treatment. Specifically, 19% of SEM and MEM patients were still seropositive 6–24 months after treatment. Comparable with our results, Philipp et al. (16) reported VlsE seropositivity in 51% (61/120) of American patients with solitary (41%, 38/93) or multiple (85%, 23/27) EMs at the initial stage and a seropositivity of 88% (105/120) during the convalescence period 1–8 weeks after presentation. The rate of seropositivity then decreased to 41% during the 6–12 months of follow-up. Tjernberg et al. (17) studied seroreactivity to VlsE protein IR_6 peptide variants and the synthetic C6 peptide in Swedish patients with SEM and found seropositivity in 66% of cases at presentation, in 64% at 2–3 months and in 44% at 6 months follow-up. Thus, in our patients the seroreactivity more often turned negative during follow-up. Differences in the seropositivity rates between the above-mentioned studies may be due to methodological differences, various criteria for positivity, timing of sampling or due to differences between European and American causative subspecies.

Thus far, no borrelia antigen has been shown to be superior to others in diagnostics of early LB. However, VlsE has been shown to be at least equally sensitive and specific compared with other borrelia antigens (18–21). It has even been suggested that 1-step tests with enzyme-linked immunoassay (ELISA) using VlsE antigen could replace the 2-step approach using ELISA followed by a confirming western blot (18, 21). In this study we used VlsE as an antigen in the confirming ELISA tests and, thus, did not use immunoblots. In addition, the 2-step approach is recommended in other forms of LB, but not in SEM (22, 23).

Remarkably, only 11/21 MEM patients tested at the initial stage were seropositive. Thus, confirmatory PCR testing of the skin lesion is needed, in clinically doubtful cases. Determination of borrelia antibodies is not recommended in case of classical SEM, with or without systemic symptoms (2), but we emphasize the need for serology in case of atypical clinical EM, resistance to adequate antibiotic therapy or in case of multiple lesions. Based on our experience of 20 years of

B. burgdorferi PCR technology, we would recommend it as a useful additional diagnostic tool. However, the method shows *B. burgdorferi* DNA only, not replicating microbes, and the DNA might remain in the tissue even long after successful treatment (24).

Post-Lyme disease and persisting symptoms after adequate treatment has been the subject of many studies (7, 24–27). In our study, a complete clinical cure of the skin lesions was achieved in all patients with SEM. In MEM, a complete cure of the skin lesions was achieved in 92% of the patients. In some patients with properly treated EM a slight hyperpigmentation may persist at the initial places of EM lesions (7), as was the case in 4 of our patients. Interestingly, in one of our patients with Sjögren's syndrome, granuloma annulare lesions developed in the areas of original EM after antimicrobial treatment. We anticipate that granuloma annulare might be triggered by LB rather than caused by LB *per se* (28–30).

The recent study by Sjöwall et al. (31) shows that a decreased Th1-type inflammatory cytokine expression in the infected skin early during the infection associates with persisting symptoms after treatment (such as arthralgia and fatigue) in 7/85 Finnish SEM patients. This could be a sign of inefficient early immune response and it is possible that some patients with autoimmune diseases may present such a deficiency. This would explain our findings of persisting skin lesions in 2 patients with autoimmune disease.

The frequency of systemic symptoms among the patients with SEM or MEM (31%) was similar to results from other European studies (27–37%) (32–34). Due to individual patient-related aspects and concomitant medications, the recommended treatment scheme sometimes varied from the guideline recommendations. The high frequency of multiple antimicrobial regimens (37%) reflects the difficulty in implementing a new treatment guideline into the healthcare system (Table V).

The prevalence of ticks carrying *B. burgdorferi* is increasing in several parts of Europe. Our retrospective analysis demonstrates that LB cannot be ruled out even if the patient does not recall a tick bite. This is in line with the observation of Stanek et al. (2) and other studies, which report a highly varying percentage (21–73%) of patients with EM recalling a tick bite (7, 31–36). It is crucial to emphasize that SEM and MEM can have varying clinical appearances (Fig. 1) and that detailed diagnostic measures are needed.

The limitations of our study are due mainly to its retrospective form. The frequency of the documentation of serological and clinical data (including photography) were not standardized in advance and therefore varied according to clinical practice of the various physicians. Furthermore, patients did not always remember the duration of the skin lesion(s). Finnish evidence-based guidelines for treatment of SEM and lymphocytoma are available, but there are no Finnish guidelines for treatment of MEM. Therefore, the treatment of MEM has been varying as listed in Table V. Antimicrobial treatments were prescribed independently also by several other physicians than dermatologists, prior to presentation in our department. In addition, subspecies of the causative agent (*B. afzelii* or *B. garinii*) were not analysed.

We conclude that EM is commonly underdiagnosed even in endemic areas. The multifaceted appearance of both SEM and MEM, combined with the fact that most patients do not recall having a tick bite, can cause difficulties in diagnosing LB in primary healthcare. In addition, the high percentage of seronegativity at the initial stage may be misleading. In this Finnish cohort of highly selected patients with PCR-confirmed LB presenting with clinically atypical SEM or MEM, more than half of the patients were not diagnosed for LB before arrival to the Department of Dermatology. This is important, since LB should be treated early, and since inadequate antimicrobial treatment might initially cause an atypical clinical picture that makes diagnosis more difficult. MEM might appear more frequently in Europe (8) than previously thought. It is likely that LB will become endemic in several parts of the world, because of climate changes that are favourable for ticks and their host animals (37). Therefore, it is important that physicians are aware of tick-borne diseases. Prospective research should be performed in order to determine whether patients with pre-existing autoimmune diseases need different treatment for LB.

ACKNOWLEDGEMENTS

The authors thank Nicolas Kluger, MD, and Leila Jeskanen, MD, for valuable comments on the manuscript and Irma Tötterman, RN, for secretarial help. The authors also thank the Helsinki University Central Hospital Research Funds and the Wilhelm and Else Stockmann Foundation, Finland for financial support.

The authors declare no conflicts of interest.

REFERENCES

1. Hubalek Z. Epidemiology of lyme borreliosis. Curr Probl Dermatol 2009; 37: 31–50.
2. Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, Krause A, et al. Lyme borreliosis: clinical case definitions for diagnosis and management in Europe. Clin Microbiol Infect 2011; 17: 69–79.
3. Hulkko T, Lyytikäinen O, Jaakola S, Kuusi M, Puumala J, Ruutu P. [Infectious diseases in Finland 2010]. [Report/webpage]. 2011; [accessed 2012 May 31]. Available from: http://www.thl.fi/thl-client/pdfs/1d73f597-8188-4ff5-b33c-101d7e1c3e90 (in Finnish).
4. Berglund J, Eitrem R, Ornstein K, Lindberg A, Ringér A, Elmrud H, et al. An epidemiologic study of Lyme disease in southern Sweden. N Engl J Med 1995; 333: 1319–1327.
5. Arnez M, Pleterski-Rigler D, Luznik-Bufon T, Ruzic-Sabljic E, Strle F. Solitary and multiple erythema migrans in child-

ren: comparison of demographic, clinical and laboratory findings. Infection 2003; 31: 404–409.
6. Constantin C, Peter O, Cerottini J, Derighetti M, Panizzon R, Guggisberg D. Erythema migrans with multiple lesions. Ann Dermatol Venereol 2000; 127: 513–516.
7. Lipsker D, Antoni-Bach N, Hansmann Y, Jaulhac B. Long-term prognosis of patients treated for erythema migrans in France. Br J Dermatol 2002; 146: 872–876.
8. Svihrova V, Hudeckova H, Jesenak M, Schwarzova K, Kostanova Z, Ciznar I. Lyme borreliosis – analysis of the trends in Slovakia, 1999–2008. Folia Microbiol (Praha) 2011; 56: 270–275.
9. Egberts F, Moller M, Proksch E, Schwarz T. Multiple erythema migrans – manifestation of systemic cutaneous borreliosis. J Dtsch Dermatol Ges 2008; 6: 350–353.
10. Åsbrink E, Hovmark A, Hederstedt B. The spirochetal etiology of acrodermatitis chronica atrophicans Herxheimer. Acta Derm Venereol 1984; 64: 506–512.
11. HUSLAB – Laboratory of Helsinki and Uusimaa Hospital District. Borrelia burgdorferi, vasta-aineet seerumista. [webpage]. 2012 [accessed 2012 May 31]. Available from: http://huslab.fi/cgi-bin/ohjekirja/tt_show.exe?assay=3552&terms=borrelia (in Finnish).
12. Ranki A, Aavik E, Peterson P, Schauman K, Nurmilaakso P. Successful amplification of DNA specific for Finnish Borrelia burgdorferi isolates in erythema chronicum migrans but not in circumscribed scleroderma lesions. J Invest Dermatol 1994; 102: 339–345.
13. Current Care working group – Finnish Medical Society Duodecim and Finnish Dermatological Society. [Bacterial infections of the skin] [webpage]. 2010 [accessed 2012 May 31]. Available from: http://www.terveysportti.fi/xmedia/hoi/hoi13020.pdf (in Finnish).
14. Uggeldahl P, Peltomaa M. [Skin manifestations of Lyme borreliosis]. Duodecim 2010; 126: 1151–1161 (in Finnish).
15. EUCALB – European concerted action on Lyme borreliosis. Treatment of Lyme borreliosis in Europe. 2009 [accessed 2012 May 31]. Available from: http://meduni09.edis.at/eucalb/cms/index.php?option=com_content&task=view&id=44&Itemid=76.
16. Philipp MT, Wormser GP, Marques AR, Bittker S, Martin DS, Nowakowski J, et al. A decline in C6 antibody titer occurs in successfully treated patients with culture-confirmed early localized or early disseminated Lyme Borreliosis. Clin Diagn Lab Immunol 2005; 12: 1069–1074.
17. Tjernberg I, Sillanpää H, Seppälä I, Eliasson I, Forsberg P, Lahdenne P. Antibody responses to borrelia IR(6) peptide variants and the C6 peptide in Swedish patients with erythema migrans. Int J Med Microbiol 2009; 299: 439–446.
18. Bacon RM, Biggerstaff BJ, Schriefer ME, Gilmore RD,Jr, Philipp MT, Steere AC, et al. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of Borrelia burgdorferi compared with 2-tiered testing using whole-cell lysates. J Infect Dis 2003; 187: 1187–1199.
19. Peltomaa M, McHugh G, Steere AC. Persistence of the antibody response to the VlsE sixth invariant region (IR6) peptide of Borrelia burgdorferi after successful antibiotic treatment of Lyme disease. J Infect Dis 2003; 187: 1178–1186.
20. Sillanpää H, Lahdenne P, Sarvas H, Arnez M, Steere A, Peltomaa M, et al. Immune responses to borrelial VlsE IR6 peptide variants. Int J Med Microbiol 2007; 297: 45–52.
21. Marangoni A, Moroni A, Accardo S, Cevenini R. Borrelia burgdorferi VlsE antigen for the serological diagnosis of Lyme borreliosis. Eur J Clin Microbiol Infect Dis 2008; 27: 349–354.
22. Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2006; 43: 1089–1134.
23. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. Lancet 2012; 379: 461–473.
24. Yrjänäinen H, Hytönen J, Hartiala P, Oksi J, Viljanen MK. Persistence of borrelial DNA in the joints of Borrelia burgdorferi-infected mice after ceftriaxone treatment. APMIS 2010; 118: 665–673.
25. Klempner M, Hu L, Evans J, Schmid C, Johnson G, Trevino R, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med 2001; 345: 85–92.
26. Sapi E, Kaur N, Anyanwu S, Luecke D, Datar A, Patel S, et al. Evaluation of in-vitro antibiotic susceptibility of different morphological forms of Borrelia burgdorferi. Infect Drug Resist 2011; 4: 97–113.
27. Embers M, Barthold S, Borda J, Bowers L, Doyle L, Hodzic E, et al. Persistence of Borrelia burgdorferi in rhesus macaques following antibiotic treatment of disseminated infection. PLoS One 2012; 7: e29914.
28. Ziemer M, Grabner T, Eisendle K, Baltaci M, Zelger B. Granuloma annulare – a manifestation of infection with Borrelia? J Cutan Pathol 2008; 35: 1050–1057.
29. Eisendle K, Zelger B. The expanding spectrum of cutaneous borreliosis. G Ital Dermatol Venereol 2009; 114: 157–171.
30. Zollinger T, Mertz K, Schmid M, Schmitt A, Pfaltz M, Kempf W. Borrelia in granuloma annulare, morphea and lichen sclerosus: a PCR-based study and review of the literature. J Cutan Pathol 2010; 37: 571–577.
31. Sjöwall J, Fryland L, Nordberg M, Sjögren F, Garpmo U, Jansson C, et al. Decreased Th1-type inflammatory cytokine expression in the skin is associated with persisting symptoms after treatment of erythema migrans. PLoS One 2011; 6: e18220.
32. Strle F, Nelson JA, Ruzic-Sabljic E, Cimperman J, Maraspin V, Lotric-Furlan S, et al. European Lyme borreliosis: 231 culture-confirmed cases involving patients with erythema migrans. Clin Infect Dis 1996; 23: 61–65.
33. Oksi J, Marttila H, Soini H, Aho H, Uksila J, Viljanen MK. Early dissemination of Borrelia burgdorferi without generalized symptoms in patients with erythema migrans. APMIS 2001; 109: 581–588.
34. Strle F, Videcnik J, Zorman P, Cimperman J, Lotric-Furlan S, Maraspin V. Clinical and epidemiological findings for patients with erythema migrans. Comparison of cohorts from the years 1993 and 2000. Wien Klin Wochenschr 2002; 114: 493–497.
35. Nadelman RB, Nowakowski J, Forseter G, Goldberg NS, Bittker S, Cooper D, et al. The clinical spectrum of early Lyme borreliosis in patients with culture-confirmed erythema migrans. Am J Med 1996; 100: 502–508.
36. Brettschneider S, Bruckbauer H, Klugbauer N, Hofmann H. Diagnostic value of PCR for detection of Borrelia burgdorferi in skin biopsy and urine samples from patients with skin borreliosis. J Clin Microbiol 1998; 36: 2658–2665.
37. Subak S. Effects of climate on variability in Lyme disease incidence in the northeastern United States. Am J Epidemiol 2003; 157: 531–538.